Exhibit B

United States Patent **6,410,264**
Crawford , et al. **June 25, 2002**

Pichia pastoris gene sequences and methods for their use

Abstract

Regulatory nucleotide sequences for a novel Pichia pastoris gene, designated PpSEC10 gene, and the nucleotide sequences and respective amino acid sequences for the secretion leader and the mature Sec10p protein components of the precursor polypeptide encoded by this novel gene are provided. These compositions are useful in methods for expression and secretion of proteins when assembled in proper reading frame, individually or in combination, within a DNA construct that further comprises a nucleotide sequence encoding a protein of interest. Vectors comprising the DNA constructs of the invention can be used to transform a yeast host cell, which can then be cultured to obtain the secreted protein of interest. Kits useful in this method and in methods of detection of the Sec10p protein using antibodies are also disclosed.

Inventors: **Crawford; Kenneth A.** (Alameda, CA); **Bishop; Robert** (San Francisco, CA)
Assignee: **Chiron Corporation** (Emeryville, CA)
Appl. No.: **127578**
Filed: **July 31, 1998**

Current U.S. Class: **435/69.1**; 435/5; 435/6; 435/91.1; 435/172.3; 435/320.1; 935/72; 935/33
Intern'l Class: C12Q 001/68
Field of Search: 935/72,33 435/69.1,6,5,91.1,172.3,320.1

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Primary Examiner: Jones; W. Gary
Assistant Examiner: Taylor; Janell E.
Attorney, Agent or Firm: Spruill; W. Murray, Guth; Joseph H., Blackburn; Robert P.

Parent Case Text

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Ser. No. 60/054783, filed Aug. 5, 1997, and U.S. Provisional Application Ser. No. 60/069560, filed Dec. 12, 1997, the contents of which are herein incorporated by reference.

Claims

That which is claimed:

1. A recombinant construct comprising in proper reading frame a nucleotide sequence for a promoter and a nucleotide sequence encoding a polypeptide, where said promoter drives transcription of an operably linked nucleotide sequence of interest, wherein said nucleotide sequence for said promoter is selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 2;

b) a nucleotide sequence having at least 70% ***sequence identity*** to the nucleotide sequence set forth in SEQ ID NO: 2;

c) a nucleotide sequence comprising at least 24 contiguous nucleotides of the sequence set forth in SEQ ID NO: 2; and

d) a nucleotide sequence that hybridizes to any one of a), b), or c) under conditions of high stringency.

2. The construct of claim 1, wherein said nucleotide sequence for said promoter is the sequence set forth in SEQ ID NO: 2.

3. The construct of claim 1, wherein said polypeptide is selected from the group consisting of human IGF-I, a polypeptide having at least 70% ***sequence identity*** to said human IGF-I, and a fragment of said human IGF-I, wherein said fragment comprises at least 10 contiguous amino acid residues of an amino acid sequence for said human IGF-I.

4. The construct of claim 1, further comprising a nucleotide sequence encoding a functional: secretion leader, wherein said nucleotide sequence encoding said secretion leader is selected from the group consisting of:

a) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 4;

b) the nucleotide sequence set forth in SEQ ID NO: 5;

c) a nucleotide sequence encoding an amino acid sequence having at least 70% ***sequence identity*** to the amino acid sequence set forth in SEQ ID NO: 4;

d) a nucleotide sequence encoding at least 8 contiguous amino acid residues of the amino acid sequence set forth in SEQ ID NO: 4; and

e) a nucleotide sequence that hybridizes to any of a), b), c), or d) under conditions of high stringency.

5. The construct of claim 4, wherein said nucleotide sequence for said promoter is the sequence set forth in SEQ ID NO: 2, and wherein said nucleotide sequence encoding said secretion leader is a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID) NO: 4.

6. The construct of claim 1, further comprising a nucleotide sequence for a functional transcription terminator, wherein said nucleotide sequence for said terminator is selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 3;

b) a nucleotide sequence having at least 70% ***sequence identity*** to the nucleotide sequence set forth in SEQ ID NO: 3;

c) a nucleotide sequence comprising at least 24 contiguous nucleotides of the sequence set forth in SEQ ID NO: 3; and

d) a nucleotide sequence that hybridizes to any one of a), b), or c) under conditions of high stringency.

7. The construct of claim 6, wherein said nucleotide sequence for said promoter is the sequence set forth in SEQ ID NO: 2, and wherein said nucleotide sequence for said terminator is the sequence set forth in SEQ ID NO: 3.

8. The construct of claim 4, further comprising a nucleotide sequence for a functional transcription terminator, wherein said nucleotide sequence for said terminator is selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 3;

b) a nucleotide sequence having at least 70% ***sequence identity*** to the nucleotide sequence set forth in SEQ ID NO: 3;

c) a nucleotide sequence comprising at least 24 contiguous nucleotides of the sequence set forth in SEQ ID NO: 3; and

d) a nucleotide sequence that hybridizes to any one of a), b), or c) under conditions of high stringency.

9. The construct of claim 8, wherein said nucleotide sequence for said promoter is the sequence set forth in SEQ ID NO: 2, and wherein said nucleotide sequence encoding said secretion leader is a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 4, and wherein said nucleotide sequence for said transcription terminator is the sequence set forth in SEQ ID NO: 3.

10. The construct of claim 8, wherein said polypeptide is selected from the group consisting of human IGF-I, a polypeptide having at least 70% ***sequence identity*** to said human IGF-I, and a fragment of said human IGF-I, wherein said fragment comprises at least 10 contiguous amino acid residues of an amino acid sequence for said human IGF-I.

11. A recombinant construct comprising in proper reading frame a nucleotide sequence for a yeast-recognized promoter, a nucleotide sequence encoding a functional secretion leader, and a nucleotide sequence encoding a polypeptide, wherein said nucleotide sequence encoding said secretion leader is selected from the group consisting of:

a) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 4;

b) the nucleotide sequence set forth in SEQ ID NO: 5;

c) a nucleotide sequence encoding an amino acid sequence having at least 70% ***sequence identity*** to the amino acid sequence set forth in SEQ ID NO: 4;

d) a nucleotide sequence encoding at least 8 contiguous amino acid residues of the amino acid sequence set forth in SEQ ID NO: 4; and

e) a nucleotide sequence that hybridizes to any of a), b), c), or d) under conditions of high stringency.

12. The construct of claim 11, wherein said nucleotide sequence encoding said secretion leader is a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 4.

13. The construct of claim 11, wherein said polypeptide is selected from the group consisting of human IGF-I, a polypeptide having at least 70% ***sequence identity*** to said human IGF-I, and a fragment of said human IGF-I, wherein said fragment comprises at least 10 continuous amino acids residues of an amino acid sequence for said human IGF-I, and wherein said nucleotide sequence encoding said secretion leader is a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 4.

14. The construct of claim 11, further comprising a nucleotide sequence for a functional transcription terminator, wherein said nucleotide sequence for said terminator is selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 3;

b) a nucleotide sequence having at least 70% ***sequence identity*** to the nucleotide sequence set forth in SEQ ID NO: 3;

c) a nucleotide sequence comprising at least 24 contiguous nucleotides of the sequence set forth in SEQ ID NO: 3; and

d) a nucleotide sequence that hybridizes to any one of a), b), or c) under conditions of high stringency.

15. The construct of claim 14, wherein said nucleotide sequence encoding said secretion leader is a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 4, and wherein said nucleotide sequence for said transcription terminator is the sequence set forth in SEQ ID NO: 3.

16. A recombinant construct comprising in proper reading frame a nucleotide sequence for a yeast-recognized promoter, a nucleotide sequence encoding a polypeptide, and a nucleotide sequence for a functional transcription terminator, wherein said nucleotide sequence for said terminator is selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 3;

b) a nucleotide sequence having at least 70% ***sequence identity*** to the nucleotide sequence set forth in SEQ ID NO: 3;

c) a nucleotide sequence comprising at least 24 contiguous nucleotides of the sequence set forth in SEQ ID NO: 3; and

d) a nucleotide sequence that hybridizes to any one of a), b), or c) under conditions of high stringency.

17. The construct of claim 16, wherein said nucleotide sequence for said terminator is the sequence set forth in SEQ ID NO: 3.

18. The construct of claim 16, wherein said polypeptide is selected from the group consisting of human IGF-I, a polypeptide having at least 70% ***sequence identity*** to said human IGF-I, and a fragment of said human IGF-I, wherein said fragment comprises at least 10 contiguous amino acids of an amino acid sequence for said human IGF-I.

19. A vector comprising at least one copy of the construct of claim 1.

20. The vector of claim 19, wherein said vector is an autonomously replicating vector.

21. The vector of claim 19, wherein said vector is an integrative vector.

22. A vector comprising at least one copy of the construct of claim 8.

23. A vector comprising at least one copy of the construct of claim 11.

24. A vector comprising at least one copy of the construct of claim 16.

25. A yeast host cell stably transformed with at least one copy of the construct of claim 1.

26. The yeast host cell of claim 25, wherein said yeast is selected from the group consisting of Pichia

United States Patent **6,384,304**
Quandt , et al. **May 7, 2002**

Conditional sterility in wheat

Abstract

The invention relates to the use of a deacetylase coding sequence for obtaining conditional sterility in wheat. The invention relates to vectors comprising a deacetylase coding sequence under control of promoters which direct stamen-selective expression in wheat, which are particularly suited for the production of wheat plants which can be made male-sterile upon application of an acetylated toxin.

Inventors: **Quandt; Jurgen** (Saskatoon, CA); **Bartsch; Klaus** (Konigstein, DE); **Knittel; Nathalie** (Frankfurt, DE)
Assignee: **Plant Genetic Systems N.V.** (Gent, BE)
Appl. No.: **418817**
Filed: **October 15, 1999**

Current U.S. Class:	**800/320.3**; 435/418; 435/419; 435/468; 800/271; 800/274; 800/278; 800/287; 800/288; 800/300; 800/303
Intern'l Class:	C12N 015/82; C12N 015/31; C12N 005/04; A01H 005/00; A01H 001/02
Field of Search:	800/320.3,288,300,287,274,303,271,278 435/418,419,468

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Primary Examiner: Fox; David T.
Attorney, Agent or Firm: Frommer Lawrence & Haug LLP

Claims

We claim:

1. A wheat plant, having a chimeric gene integrated into its genome, the chimeric gene comprising:

a) a DNA molecule encoding a deacetylase from Stenotrophomonas sp; and

b) a promoter directing stamen-selective expression in wheat wherein the DNA molecule encoding the deacetylase is in the same transcriptional unit and under control of the stamen-selective promoter.

2. The wheat plant of claim 1, wherein said DNA molecule encodes a biologically active fragment or a variant of the deacetylase encoded by SEQ ID No. 9.

3. The wheat plant of claim 1, wherein said DNA molecule encodes a deacetylase having the amino acid sequence of SEQ ID No. 8.

4. The wheat plant of claim 1, wherein said DNA molecule comprises the sequence of SEQ ID No. 9, or a sequence capable of hybridizing to SEQ ID No. 9 under standard ***stringent*** conditions.

5. The wheat plant of claim 1, wherein said stamen-selective promoter is a CA55 promoter.

6. The wheat plant of claim 1, wherein said stamen-selective promoter is a T72 promoter.

7. The wheat plant of claim 1, wherein said stamen-selective promoter is an E1 promoter.

8. A process for producing ***hybrid*** wheat seed, said process comprising

i) producing seeds capable of growing into conditionally male-sterile wheat plants, said seeds having a chimeric gene integrated in their genome, the chimeric gene comprising:

a) a DNA molecule encoding a deacetylase from Stenotrophomonas sp., and

b) a stamen-selective promoter wherein the DNA molecule encoding the deacetylase is in the same transcriptional unit and under the control of the stamen-selective promoter;

ii) interplanting said seeds capable of growing into conditionally male-sterile wheat plants with seeds capable of growing into male fertile wheat plants;

iii) inducing male-sterility in said conditionally male-sterile plants by applying an N-acetyl-PPT, which in itself is not toxic to the plants or plant cells; and

iv) harvesting ***hybrid*** seed.

9. The process of claim 8, wherein said DNA molecule encodes a biologically active fragment or a variant of the deacetylase encoded by SEQ. ID. No. 9.

10. The process of claim 8, wherein said DNA molecule encodes the deacetylase comprising the amino acid sequence of SEQ ID No. 8.

11. The process of claim 8, wherein said DNA molecule comprises the sequence of SEQ ID No. 9, or a sequence capable of hybridizing to SEQ ID No. 9 under standard ***stringent*** conditions.

12. The process of claim 8, wherein said stamen-selective promoter is a CA55 promoter.

13. The process of claim 8, wherein said stamen-selective promoter is a T72 promoter.

14. The process of claim 8, wherein said stamen-selective promoter is an E1 promoter.

15. The process of claim 8, wherein said male fertile plants are female-sterile.

16. A process for producing a conditionally male-sterile wheat plant, said process comprising

i) transforming a wheat plant cell or tissue with a chimeric gene which comprises:

a) a DNA molecule encoding a deacetylase from Stenotrophomonas sp., and

b) a stamen-selective promoter wherein the DNA molecule encoding the deacetylase is in the same transcriptional unit and under the control of said stamen-selective promoter;

ii) regenerating said conditionally male-sterile plant from said cell or tissue; and optionally,

iii) applying an N-acetyl-PPT to said conditionally male-sterile plant, which is in itself not toxic to the plant or plant cells to make said plant male-sterile.

17. The process of claim 16, wherein said DNA molecule encodes a biologically active fragment of the deacetylase encoded by SEQ. ID. No. 9.

18. The process of claim 16, wherein said DNA molecule encodes the deacetylase of SEQ ID No. 8.

19. The process of claim 16, wherein said DNA molecule comprises the sequence of SEQ ID No. 9, or a sequence capable of hybridizing to SEQ ID No. 9 under standard ***stringent*** conditions.

20. The process of claim 16, wherein said stamen-selective promoter is a CA55 promoter.

21. The process of claim 16, wherein said stamen-selective promoter is a T72 promoter.

22. The process of claim 16, wherein said stamen-selective promoter is an E1 promoter.

23. A conditionally male sterile wheat plant obtained by the process of claim 16.

24. A process for generating male sterility in wheat plants, said process comprising

i) obtaining a conditionally male-sterile wheat plant by transforming a wheat plant cell or tissue with a chimeric gene which comprises:

a) a DNA molecule encoding a deacetylase from Stenotrophomonas sp., and

b) a stamen-selective promoter wherein the DNA molecule encoding the deacetylase is in the same transcriptional unit and under the control of said stamen-selective promoter, and regenerating said conditionally male-sterile plant from said cell or tissue; and optionally obtaining conditionally male-sterile progeny from said plant

ii) applying an N-acetyl-PPT to said conditionally male-sterile plant or its conditionally male-sterile progeny, which is in itself not toxic to the plant or plant cells to make said plant male-sterile.

25. A male sterile wheat plant, or cells or tissues thereof, obtained by the process of 24.

26. Wheat plant cells, tissues or seed, each transformed with the chimeric DNA of claim 1.

United States Patent **6,417,428**
Thomashow , et al. **July 9, 2002**

Plant having altered environmental stress tolerance

Abstract

A transformed plant is provided which comprises one or more environmental stress tolerance genes; a DNA regulatory sequence which regulates expression of the one or more environmental stress tolerance genes; a sequence encoding a binding protein capable of binding to the DNA regulatory sequence and inducing expression of the one or more environmental stress tolerance genes; and a recombinant promoter which regulates expression of the gene encoding the binding protein. A method for altering an environmental stress tolerance of a plant is also provided which comprises the steps of transforming a plant with a promoter which regulates expression of at least one copy of a gene encoding a binding protein capable of binding to a DNA regulatory sequence which regulates one or more environmental stress tolerance genes in the plant; expressing the binding protein encoded by the gene; and stimulating expression of at least one environmental stress tolerance gene through binding of the binding protein to the DNA regulatory sequence.

Inventors: **Thomashow; Michael F.** (805 Southlawn, East Lansing, MI 48823); **Stockinger; Eric J.** (1360 Burcham Dr., East Lansing, MI 48823); **Jaglo-Ottosen; Kirsten** (307 S. Clemens Ave., Lansing, MI 48912); **Gilmour; Sarah Jane** (1830 Barnes Rd., Leslie, MI 49251); **Zarka; Daniel** (2101 Barritt St., Lansing, MI 48912); **Jiang; Cai-Zhong** (1301 Orchard Park Cir., Apt. C-4, Davis, CA 94516)

Appl. No.: **198119**
Filed: **November 23, 1998**

Current U.S. Class: **800/260**; 435/6; 536/22.1; 536/23.1
Intern'l Class: A01H 001/00; C12Q 001/68; C07H 019/00
Field of Search: 435/6 536/22.1,23.1 800/205

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Primary Examiner: Riley; Jezia
Attorney, Agent or Firm: Guerrero; Karen

Goverment Interests

The US government has rights to the present invention under grants from the USDA/NRICGP-.

Parent Case Text

RELATIONSHIP TO COPENDING APPLICATIONS

This application is a continuation-in-part of the following U.S. applications: U.S. application Ser. No.: 09/018,233, filed: Feb. 3, 1998 entitled "ISOLATED DNA ENCODING ENVIRONMENTAL STRESS TOLERANCE REGULATORY BINDING PROTEIN;" now abandoned U.S. application Ser. No.: 09/017,816, filed: Feb. 3, 1998 entitled "CONSTRUCT FOR TRANSFORMING CELL WITH SEQUENCE ENCODING ENVIRONMENTAL STRESS TOLERANCE REGULATORY BINDING PROTEIN;" now abandoned U.S. application Ser. No.: 09/018,235, filed: Feb. 3, 1998 entitled

"ENVIRONMENTAL STRESS TOLERANCE REGULATORY BINDING PROTEIN TRANSFORMED CELL EXPRESSING ENVIRONMENTAL;" now abandoned U.S. application Ser. No.: 09/017,575 filed: Feb. 3, 1998 entitled "STRESS TOLERANCE REGULATORY BINDING PROTEIN;" now abandoned U.S. application Ser. No.: 09/018,227, filed: Feb. 3, 1998 entitled "TRANSFORMED PLANT WITH MODIFIED ENVIRONMENTAL STRESS TOLERANCE GENE EXPRESSION;" now abandoned U.S. application Ser. No.: 09/018,234, filed: Feb. 3, 1998 entitled "METHOD FOR REGULATING EXPRESSION OF STRESS TOLERANCE GENES IN A TRANSFORMED PLANT;" now abandoned and U.S. application Ser. No.: 08/706,270; filed: Sep. 4, 1996, entitled "now U.S. Pat. No. 5,892,009, " each of which are incorporated herein by reference.

Claims

We claim:

1. A plant comprising a recombinant molecule comprising a polynucleotide that encodes a polypeptide comprising an AP2 domain having at least an 82% ***sequence identity*** to an AP2 domain from the group of sequences consisting of SEQ ID Nos:13, 15, 17, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93 and 95.

2. The plant of claim 1, wherein said polypeptide binds to a cold or dehydration transcription regulating region comprising the sequence CCG.

3. The plant of claim 1, wherein said polypeptide binds to a member of a class of DNA regulatory sequences which includes a subsequence selected from the group consisting of CCGAA, CCGAT, CCGAC, CCGAG, CCGTA, CCGTT, CCGTC, CCGTG, CCGCA, CCGCT, CCGCG, CCGCC, CCGGA, CCGGT, CCGGC, CCGGG, AACCG, ATCCG, ACCCG, AGCCG, TACCG, TTCCG, TCCCG, TGCCG, CACCG, CTCCG, CGCCG, CCCCG, GACCG, GTCCG, GCCCG, GGCCG, ACCGA, ACCGT, ACCGC, ACCGG, TCCGA, TCCGT, TCCGC, TCCGG, CCCGA, CCCGT, CCCGC, CCCGG, GCCGA, GCCGT, GCCGC, and GCCGG.

4. The plant of claim 1, wherein said recombinant molecule comprises a polynucleotide encoding a polypeptide that elevates cold-regulated gene levels in the absence of cold acclimation compared with cold-regulated gene levels in a plant lacking said recombinant molecule.

5. The plant of claim 1, further comprising a promoter and wherein said polynucleotide is expressed under regulatory control of the promoter.

6. The plant of claim 5, wherein said promoter is regulated by the addition of an exogenous agent.

7. The plant of claim 5, wherein said promoter is a constitutive promoter.

8. The plant of claim 5, wherein said promoter is regulated by changes in environment conditions.

9. A plant comprising a recombinant molecule comprising a polynucleotide that hybridizes to the AP2 domain of a member of the group of sequences consisting of SEQ ID Nos:12, 14, 18, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92 and 94 under high stringency conditions.

10. The plant of claim 9 comprising a recombinant polynucleotide comprising a sequence that

hybridizes to a member of the group of sequences consisting of SEQ ID Nos: 12, 14, 18, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92 and 94 under high stringency conditions.

11. A method for altering an environmental stress response of a plant, said method comprising (a) providing a recombinant molecule comprising a polynucleotide that encodes a polypeptide comprising an AP2 domain having at least an 82% ***sequence identity*** to an AP2 domain selected from the group of sequences consisting of SEQ ID Nos:2, 13, 15, 17, 39, 41, 43, 45,47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93 and 95; and (b) introducing said recombinant molecule into the plant.

12. The method of claim 11, wherein said polypeptide binds to a cold or dehydration transcription regulating region comprising the sequence CCG.

13. The method of claim 12, wherein said polypeptide binds to a member of a class of DNA regulatory sequences which includes a subsequence selected from the group consisting of CCGAA, CCGAT, CCGAC, CCGAG, CCGTA, CCGTT, CCGTC, CCGTG, CCGCA, CCGCT, CCGCG, CCGCC, CCGGA, CCGGT, CCGGC, CCGGG, AACCG, ATCCG, ACCCG, AGCCG, TACCG, TTCCG, TCCCG, TGCCG, CACCG, CTCCG, CGCCG, CCCCG, GACCG, GTCCG, GCCCG, GGCCG, ACCGA, ACCGT, ACCGC, ACCGG, TCCGA, TCCGT, TCCGC, TCCGG, CCCGA, CCCGT, CCCGC, CCCGG, GCCGA, GCCGT, GCCGC, and GCCGG.

14. The method of claim 11, wherein said recombinant polynucleotide comprises a sequence encoding a polypeptide that elevates cold-regulated gene levels in the absence of cold acclimation compared with cold-regulated gene levels in a plant lacking said recombinant molecule.

15. The method of claim 11, further comprising a promoter and wherein said polynucleotide is expressed under regulatory control of the promoter.

16. The method of claim 15, wherein said promoter is regulated by the addition of an exogenous agent.

17. The method of claim 15, wherein said promoter is a constitutive promoter.

18. The method of claim 15, wherein said promoter is regulated by changes in environment conditions.

19. A method for altering an environmental stress response of a plant, comprising (a) providing a recombinant molecule comprising a polynucleotide that encodes a polypeptide comprising a polynucleotide that hybridizes to a member of the group of sequences consisting of SEQ ID Nos: 1, 12, 14, 18, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92 and 94 under high stringency conditions; and (b) introducing said recombinant molecule into the plant.

20. A recombinant molecule comprising a polynucleotide that encodes a polypeptide comprising an AP2 domain having at least an 82% sequence identity to an AP2 domain selected from the group of sequences consisting of SEQ ID Nos: 13, 15, 17, 39, 41, 43, 45, 47,49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93 and 95.

21. The recombinant molecule of claim 20, wherein said polypeptide binds to a cold or dehydration transcription regulating region comprising the sequence CCG.

22. The recombinant molecule of claim 21, wherein said polypeptide binds to a member of a class of

DNA regulatory sequences which includes a subsequence selected from the group consisting of CCGAA, CCGAT, CCGAC, CCGAG, CCGTA, CCGTT, CCGTC, CCGTG, CCGCA, CCGCT, CCGCG, CCGCC, CCGGA, CCGGT, CCGGC, CCGGG, AACCG, ATCCG, ACCCG, AGCCG, TACCG, TTCCG, TCCCG, TGCCG, CACCG, CTCCG, CGCCG, CCCCG, GACCG, GTCCG, GCCCG, GGCCG, ACCGA, ACCGT, ACCGC, ACCGG, TCCGA, TCCGT, TCCGC, TCCGG, CCCGA, CCCGT, CCCGC, CCCGG, GCCGA, GCCGT, GCCGC, and GCCGG.

23. The recombinant molecule of claim 20, wherein said recombinant polynucleotide comprises a sequence encoding a polypeptide that elevates cold-regulated gene levels in the absence of cold acclimation compared with cold-regulated gene levels in a plant lacking said recombinant molecule.

24. The recombinant molecule of claim 20, further comprising a promoter and wherein said polynucleotide is expressed under regulatory control of the promoter.

25. The recombinant molecule of claim 24, wherein said promoter is regulated by the addition of an exogenous agent.

26. The recombinant molecule of claim 24, wherein said promoter is a constitutive promoter.

27. The recombinant molecule of claim 24, wherein said promoter is regulated by changes in environment conditions.

28. A recombinant molecule comprising a polynucleotide that hybridizes to the AP2 domain of a member of the group of sequences consisting of SEQ ID Nos:12, 14, 18, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92 and 94 under high stringency conditions.

29. The method of claim 11, wherein said environmental stress response is a response to cold or freezing.

30. The method of claim 11, wherein said environmental stress response is a response to drought.

31. The method of claim 11, wherein said environmental stress response is a response to salinity.

32. The plant of claim 1, wherein said recombinant molecule comprises a polynucleotide that encodes a polypeptide comprising an AP2 domain having at least an 82% ***sequence identity*** to an AP2 domain from the group of sequences consisting of SEQ ID Nos:13, 15, 17, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93 and 95.

33. The method plant of claim 11, wherein said recombinant molecule comprises a polynucleotide that encodes a polypeptide comprising an AP2 domain having at least an 82% ***sequence identity*** to an AP2 domain from the group of sequences consisting of SEQ ID Nos:13, 15, 17, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93 and 95.

United States Patent **6,413,522**
De Villiers-Zur Hausen , et al. **July 2, 2002**

Papilloma viruses, products for the detection thereof as well as for treating diseases caused by them

Abstract

This invention relates to a DNA coding for a peptide of a papilloma virus major capsid protein and a papilloma virus genome, respectively. Furthermore, this invention concerns proteins coded by the papilloma virus genome and antibodies directed thereagainst as well as the use thereof for diagnosis, treatment and vaccination.

Inventors: **De Villiers-Zur Hausen; Ethel-Michele** (Waldmichelbach, DE); **Zur Hausen; Harald** (Waldmichelbach, DE); **Lavergne; Donna** (Florsheim/Dalsheim, DE); **Benton; Claire** (Edinburgh, GB)
Assignee: **Deutsches Krebsforschungszentrum Stiftung des Offentlichen Rechts** (Heidelberg, DE)

Appl. No.: **319056**
Filed: **August 5, 1999**
PCT Filed: **November 12, 1997**
PCT NO: **PCT/DE97/02659**
371 Date: **August 5, 1999**
102(e) Date: **August 5, 1999**
PCT PUB.NO.: **WO98/23752**
PCT PUB. Date: **June 4, 1998**

Foreign Application Priority Data

Nov 26, 1996[DE] 196 48 962

Current U.S. Class: **424/204.1**; 424/199.1; 435/5; 435/69.1; 435/69.3; 435/235.1; 435/320.1; 536/23.72; 530/350; 530/324
Intern'l Class: A61K 039/12; C12N 001/12; C12N 015/00; C07H 021/04
Field of Search: 435/6,701,69.3,69.1,235.1,320.1 536/23.1,23.72 396/2,1 530/350,387.1,324 424/204.1,199.1

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Primary Examiner: Salimi; Ali R.
Attorney, Agent or Firm: Pennie & Edmonds LLP

Parent Case Text

This is a national phase filing of the Application No. PCT/DE97/02659, which was filed with the Patent Corporation Treaty on Nov. 12, 1997, and is entitled to priority of the German Patent Application DE 196 48 962.8, filed Nov. 26, 1996.

Claims

What is claimed is:

1. An isolated polynucleotide consisting essentially of:

(a) a nucleotide sequence encoding the peptide of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:8;

(b) a nucleotide sequence hybridizing to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7; or

(c) the complement of (a) or (b);

wherein the polynucleotide has a homology of at least 90% to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7, or the complement thereof.

2. An isolated polynucleotide encoding a peptide of a papilloma virus major capsid protein, wherein the said polynucleotide has been obtained using the following steps:

(a) incubating total DNA isolated from a biopsy of epithelial neoplasm with a nucleic acid having at least a portion of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7, under a condition that allows hybridization of a polynucleotide derived from a papilloma virus genome included in the total DNA to said nucleotide sequence of the complement of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7; and

(b) identifying and isolating a polynucleotide that hybridizes to the complement of the nucleotide

sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7 in step (a);

wherein the polynucleotide has a homology of at least 90% to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7.

3. An isolated polynucleotide, consisting essentially of (a) a nucleic acid encoding a peptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:8, or (b) the complement of (a).

4. An isolated polynucleotide, wherein the polynucleotide consists essentially of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7, or the complement thereof.

5. A plasmid comprising the polynucleotide of claim 1 or 2.

6. A plasmid comprising the polynucleotide of claim 3 or 4.

7. An expression vector comprising the polynucleotide of claim 1 or 2.

8. An expression vector comprising the polynucleotide of claim 3 or 4.

9. A host cell comprising the plasmid of claim 5.

10. A host cell comprising the plasmid of claim 6.

11. A host cell comprising the expression vector of claim 9.

12. A host cell comprising the expression vector of claim 8.

13. A method of producing a peptide of a papilloma virus major capsid protein, comprising cultivating the host cell of claim 11 under suitable conditions.

14. A method of producing a peptide of a papilloma virus major capsid protein, comprising cultivating the host cell of claim 12 under suitable conditions.

15. A method of detecting a papilloma virus DNA, comprising:

(a) ***hybridizing under stringent conditions*** at least a portion of the polynucleotide of claim 1, 2, 3, or 4 to a DNA sample; and

(b) identifying papilloma virus in said DNA sample by detecting a hybridization signal.

16. A composition comprising the polynucleotide of claim 1, 2, 3, or 4 as reagent for diagnosis and a diagnostically acceptable carrier.

17. A method of producing a papilloma virus genome, comprising:

(a) incubating total DNA isolated form a biopsy of epithlial neoplasm with a nucleic acid having at least a portion of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7, under a condition that allows hybridization of a polynucleotide derived from a papilloma virus genome included in the total DNA to said nucleotide sequence of the complement of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7; and

(b) identifying and isolating a polypnucleotide that hybridizes to the nucleotide sequence of the complement of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7 in step (a).

18. The method of claim 17, wherein the polypnucleotide has a homology of at least 90% to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7.

19. A composition comprising the polynucleotide of claim 1, 2, 3, or 4 as reagent for vaccination and a pharmaceutically acceptable carrier.

20. A method of vaccinating a subject in need against papilloma virus, comprising administering to said subject the composition of claim 19.

21. A method of diagnosing a condition caused by papilloma virus in a subject in need, comprising exposing said subject the composition of claim 16.

22. A method of using the polynucleotide of claim 1, 2, 3, or 4 as reagent for diagnosis.

23. The method according to claim 22, wherein the diagnosis concerns papilloma virus infections or diseases.

United States Patent **6,372,961**
Tarczynski , et al. **April 16, 2002**

Hemoglobin genes and their use

Abstract

The invention relates to the genetic manipulation of plants, particularly to the expression of hemoglobin genes in transformed plants. Nucleotide sequences for the hemoglobin genes and methods for their use are provided. The sequences find use in enhancing seed germination, seedling growth, and overall growth and metabolism of the plant.

Inventors: **Tarczynski; Mitchell C.** (West Des Moines, IA); **Shen; Bo** (Johnston, IA)
Assignee: **Pioneer Hi-Bred International, Inc.** (Des Moines, IA)
Appl. No.: **376728**
Filed: **August 17, 1999**

Current U.S. Class:	**800/278**; 435/6; 435/91.1; 435/320.1; 435/419; 435/468; 536/23.1; 800/295; 800/300.1; 800/306
Intern'l Class:	C12Q 001/68; A01H 005/00; C12N 005/14; C12N 005/82; C07H 021/04
Field of Search:	435/6,320.1,91.1,419,468 536/23.1,24.5,24.3,24.31,24.33 800/295,278,300.1

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Primary Examiner: Wang; Andrew
Assistant Examiner: Lacourciere; Karen A
Attorney, Agent or Firm: Pioneer Hi-Bred International, Inc.

Parent Case Text

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. application Ser. No. 60/097,242 filed Aug. 20, 1998, which is herein incorporated by reference.

Claims

That which is claimed:

1. An isolated nucleotide sequence selected from the group consisting of:

A) a nucleotide sequence encoding the amino acid sequence of SEQ ID NOS: 2 or 4;

B) a nucleotide sequence set forth in SEQ ID NOS: 1 or 3;

C) a polynucleotide encoding a hemoglobin polypeptide, said polynucleotide having at least 70 percent ***sequence identity*** to SEQ ID NOS: 1 or 3, wherein the percent ***sequence identity*** is based on the entire sequence and is determined by GAP analysis using default parameters; and

D) a polynucleotide complementary to the entire length of a polynucleotide of (A) through, (C).

2. An expression cassette comprising a nucleotide sequence of claim 1, wherein said nucleotide sequence is operably linked to a promoter that drives expression in a plant cell.

3. The expression cassette of claim 2, wherein said promoter is a tissue specific promoter.

4. The expression cassette of claim 3, wherein said promoter is selected from the group consisting of promoters driving expression in root, seed, embryo, and green tissue.

5. The expression cassette of claim 2, wherein said promoter is a constitutive promoter.

6. The expression cassette of claim 2, wherein said cassette further comprises a chloroplast targeting sequence operably linked to the nucleotide sequence.

7. The expression cassette of claim 6, wherein said promoter is a constitutive promoter.

8. A method for enhancing seed germination and seedling growth, said method comprising transforming a plant with at least one nucleotide sequence encoding a hemoglobin protein said nucleotide sequence operably linked to a promoter that drives expression in a plant cell, wherein said nucleotide sequence is selected from the group consisting of:

A) a nucleotide sequence encoding the amino acid sequence of SEQ ID NOS: 2 or 4;

B) a nucleotide sequence set forth in SEQ ID NOS: 1 or 3;

C) a polynucleotide having at least 70 percent ***sequence identity*** to SEQ ID NOS: 1 or 3, wherein the percent ***sequence identity*** is based on the entire sequence and is determined by GAP analysis using default parameters; and

D) a polynucleotide complementary to the entire length of a polynucleotide of (A) through, (C).

9. The method of claim 8, wherein said promoter is a seed-specific or an embryo-specific promoter.

10. The method of claim 9, wherein said promoter is an alpha-amylase promoter.

11. A method for manipulating oxygen concentration in a plant cell, said method comprising transforming said plant cell with at least one nucleotide sequence encoding a hemoglobin protein said nucleotide sequence operably linked to a promoter that drives expression in a plant cell, wherein said nucleotide sequence is selected from the group consisting of:

A) a nucleotide sequence encoding the amino acid sequence of SEQ ID NOS: 2 or 4;

B) a nucleotide sequence set forth in SEQ ID NOS: 1 or 3;

C) a polynucleotide having at least 70 percent ***sequence identity*** to SEQ ID NOS: 1 or 3, wherein the percent ***sequence identity*** is based on the entire sequence and is determined by GAP analysis using default parameters; and

D) a polynucleotide complementary to the entire length of a polynucleotide of (A) through (C).

12. The method of claim 11, wherein said promoter is a constitutive promoter.

13. A transformed plant cell having stably incorporated into its genome at least one nucleotide sequence encoding a hemoglobin protein said nucleotide sequence operably linked to a promoter that drives expression in a plant cell, wherein said nucleotide sequence is selected from the group consisting of:

a) a nucleotide sequence encoding the amino acid sequence of SEQ ID NOS: 2 or 4;

b) a nucleotide sequence set forth in SEQ ID NOS: 1 or 3;

c) a polynucleotide having at least 70 percent ***sequence identity*** to SEQ ID NOS: 1 or 3, wherein the percent ***sequence identity*** is based on the entire sequence and is determined by GAP analysis using default parameters; and

d) a polynucleotide complementary to the entire length of a polynucleotide of (A) through (C);

and wherein said transformed plant cell has been transformed with said nucleotide sequence.

14. The plant cell of claim 13, wherein said promoter is a seed-specific or an embryo-specific promoter.

15. The plant cell of claim 13, wherein said promoter is an alpha-amylase promoter.

16. The plant cell of claim 15, wherein said promoter is a constitutive promoter.

17. A transformed plant having stably incorporated into its genome at least one nucleotide sequence encoding a hemoglobin protein said nucleotide sequence operably linked to a promoter that drives expression in a plant cell, wherein said nucleotide sequence is selected from the group consisting of:

a) a nucleotide sequence encoding the amino acid sequence of SEQ ID NOS: 2 or 4;

b) a nucleotide sequence set forth in SEQ ID NOS: 1 or 3;

c) a polynucleotide having at least 70 percent ***sequence identity*** to SEQ ID NOS: 1 or 3, wherein the percent ***sequence identity*** is based on the entire sequence and is determined by GAP analysis using default parameters; and

d) a polynucleotide complementary to the entire length of a polynucleotide of (A) through (C);

and wherein said transformed plant has been transformed with said nucleotide sequence.

18. The plant of claim 17, wherein said promoter is a seed-specific or an embryo-specific promoter.

19. The plant of claim 17, wherein said promoter is an alpha-amylase promoter.

20. The plant of claim 17, wherein said promoter is a constitutive promoter.

21. The plant of claim 17, wherein said plant is a monocot.

22. The plant of claim 21, wherein said monocot is corn, wheat, rice, barley, sorghum, or rye.

23. The plant of claim 17, wherein said plant is a dicot.

24. The plant of claim 23, wherein said dicot is selected from the group consisting of soybean, non-vegetable brassica, sunflower, alfalfa, cotton or safflower.

25. Seed of the plant of claim 21.

26. Seed of the plant of claim 22.

27. Seed of the plant of claim 23.

28. Seed of the plant of claim 24.

29. A method for modulating hemoglobin levels in a plant cell, said method comprising transforming said plant cell with at least one nucleotide sequence encoding a hemoglobin protein, said nucleotide sequence operably linked to a promoter in a plant cell, wherein said nucleotide sequence is selected from the group consisting of:

A) a nucleotide sequence encoding the amino acid sequence of SEQ ID NOS: 2 or 4;

B) a nucleotide sequence set forth in SEQ ID NOS: 1 or 3;

C) a polynucleotide having at least 70 percent ***sequence identity*** to SEQ ID NOS: 1 and 3, wherein the percent ***sequence identity*** is based on the entire sequence and is determined by GAP analysis using default parameters; and

D) a polynucleotide complementary to the entire length of a polynucleotide of (A) through (C).

30. The method of claim 29, wherein said plant cell is maize.

31. The method of claim 29, wherein said promoter is a heterologous promoter.

32. The method of claim 29, wherein said promoter is a constitutive promoter.

33. An isolated nucleotide sequence having at least 50 nucleotides in length which hybridizes under high stringency conditions, including a wash in 0.1.times.SSC to 60 to 65.degree. C., to a polynucleotide having the sequence set forth in SEQ ID NOS: 1 or 3.

United States Patent **6,274,790**
Kunst , et al. **August 14, 2001**

Nucleic acids encoding a plant enzyme involved in very long chain fatty acid synthesis

Abstract

Nucleic acid molecules encoding an enzyme involved in very long chain fatty acid (VLCFA) elongation in plants are disclosed. The invention includes a cDNA, genomic clone and encoded protein, as well as plants having modified VLCFA composition, such as modified epicuticular waxes, and methods of making such plants.

Inventors: **Kunst; Ljerka** (North Vancouver, CA); **Millar; Anthony A.** (Vancouver, CA)
Assignee: **The University of British Columbia** (Vancouver, CA)
Appl. No.: **058947**
Filed: **April 10, 1998**

Current U.S. Class: **800/287**; 435/468; 536/24.1; 800/281; 800/298
Intern'l Class: C12N 015/82; A01H 005/00; C07H 021/04
Field of Search: 800/298,281,264,287 435/69.1,468,419,430,320.1 536/23.6

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Primary Examiner: McElwain; Elizabeth F.
Attorney, Agent or Firm: Klarquist Sparkman Campbell Leigh & Whinston, LLP

Parent Case Text

PRIORITY CLAIM

This application claims priority to co-pending U.S. provisional patent application Ser. No. 60/043,831, filed on Apr. 14, 1997.

Claims

We claim:

1. A recombinant nucleic acid molecule comprising a promoter sequence operably linked to a nucleic acid sequence, wherein the promoter sequence comprises a transcriptional regulatory region capable of mediating gene expression in epidermal cells of Arabidopsis wherein the transcriptional regulatory region hybridezes under stringent conditions to: Seq. I.D. No. 12 or the complement of Seq. I.D. No. 12.

2. A recombinant nucleic acid molecule according to claim 1 wherein the promoter sequence comprises at least 50 consecutive nucleotides of the sequence shown in Seq. I.D. No. 12 or the complement of Seq. I.D. No. 12.

3. The recombinant nucleic acid molecule according to claim 1, wherein the promoter sequence is at least 70% identical to the sequence set forth in Seq. I.D. No. 12.

4. A recombinant nucleic acid molecule according to claim 1, wherein the promoter sequence is at least 80% identical with the sequence set forth in Seq. I.D. No. 12 or the complement of Seq. I.D. No. 12.

5. A recombinant vector comprising a nucleic acid molecule according to claim 1.

6. A transgenic plant comprising a heterologous nucleic acid sequence, wherein the heterologous nucleic acid sequence comprises the recombinant nucleic acid molecule of claim 1.

7. The recombinant nucleic acid molecule according to claim 1, wherein the nucleic acid sequence encodes a protein having very long chain fatty acid elongase activity.

8. A method of producing a transgenic plant comprising introducing into the plant the recombinant nucleic acid molecule of claim 1.

9. A plant produced by sexual or asexual propagation of the transgenic plant produced according to the method of claim 8, or by propagation of progeny of the transgenic plant, wherein the plant comprises the recombinant nucleic acid molecule.

10. A method of isolating a nucleic acid molecule having promoter activity, comprising ***hybridizing under stringent conditions*** a nucleic acid preparation with a probe comprising Seq. I.D. No. 12 or the complement of Seq. I.D. No. 12.

11. A plant cell comprising a heterologous nucleic acid sequence, wherein the heterologous nucleic acid sequence comprises the recombinant nucleic acid molecule of claim 1.

12. A recombinant nucleic acid molecule comprising a promoter sequence operably linked to a nucleic acid sequence, wherein the promoter sequence comprises a transcriptional regulatory region capable of mediating gene expression in epidermal cells of Arabidopsis wherein the transcriptional regulatory region is obtainable from a plant VLCFA condensing enzyme gene comprising an open reading frame that hybridizes under stringent conditions to Seq. I.D. No. 3 or to the complement of Seq. I.D. No. 3.

United States Patent **6,413,752**
Takashima , et al. **July 2, 2002**

Protein capable of catalyzing transamination stereoselectively, gene encoding said protein and use thereof

Abstract

A novel protein from Mycobacterium aurum SC-D423 capable of converting acetophenone to an optically active 1-phenylethylamine in the presence of a racemic mixture of sec-butylamine is provided.

Inventors: **Takashima; Yoshiki** (Nishinomiya, JP); **Mitsuda; Satoshi** (Takarazuka, JP); **Wieser; Marco** (Mino, JP)
Assignee: **Sumitomo Chemical Company, Limited** (Osaka, JP)

Appl. No.: **527522**
Filed: **March 16, 2000**

Foreign Application Priority Data

Mar 19, 1999[JP] 11-075511
Mar 30, 1999[JP] 11-088634

Current U.S. Class: **435/193**
Intern'l Class: C12N 009/10
Field of Search: 435/193

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Primary Examiner: Slobodyansky; Elizabeth
Attorney, Agent or Firm: Sughrue Mion, PLLC

Claims

What is claimed is:

1. An isolated protein comprising an amino acid sequence represented by SEQ ID NO. 1, wherein said protein converts acetophenone to an optically active 1-phenylethylamine in the presence of a racemic mixture of sec-butylamine.

2. An isolated protein comprising an amino acid sequence represented by SEQ ID NO. 1, wherein said protein converts acetophenone to an optically active 1-phenylethylamine in the presence of a racemic mixture of sec-butylamine, and wherein said protein has a single amino acid substitution within Sequence ID No. 1.

3. The isolated protein of claim 2, wherein alanine is substituted for threonine at position number 2 in SEQ ID NO. 1.

4. An isolated protein comprising an amino acid sequence which has at least 60% ***sequence identity*** to SEQ ID NO. 1, wherein said protein converts acetophenone to an optically active 1-phenylethylamine in the presence of a racemic mixture of sec-butylamine and wherein said protein has a molecular weight of about 37 kDa as a monomer, and is obtainable from Mycobacterium aurum SC-S432.

5. An isolated protein comprising an amino acid sequence which has at least 90% ***sequence identity*** to SEQ ID NO. 1, wherein said protein converts acetophenone to an optically active 1-phenylethylamine in the presence of a racemic mixture of sec-butylamine.

6. The isolated protein of claim 5, wherein said protein has at least 95% ***sequence identity*** to SEQ ID NO. 1.

7. An isolated protein comprising an amino acid sequence which has at least 80% ***sequence identity*** to SEQ ID NO. 1, wherein said protein converts acetophenone to an optically active 1-phenylethylamine in the presence of a racemic mixture of sec-butylamine, and wherein said protein has a molecular weight of about 37 kDa as a monomer.

8. The isolated protein of claim 7, wherein said protein has at least 90% ***sequence identity*** to SEQ ID NO. 1.

9. The isolated protein of claim 8, wherein said protein has at least 95% ***sequence identity*** to SEQ ID NO. 1.

10. An isolated protein comprising an amino acid sequence which has at least 80% ***sequence identity*** to SEQ ID NO. 1, wherein said protein converts acetophenone to an optically active 1-phenylethylamine in

the presence of a racemic mixture of sec-butylamine, and wherein said protein is derived from a microorganism belonging to the genus Mycobacterium.

11. The isolated protein of claim 10, wherein said protein has at least 90% ***sequence identity*** to SEQ ID NO. 1.

12. The isolated protein of claim 11, wherein said protein has at least 95% ***sequence identity*** to SEQ ID NO. 1.

13. An isolated protein comprising an amino acid sequence represented by amino acids 23 to 339 of SEQ ID NO. 1, wherein said protein converts acetophenone to an optically active 1-phenylethylamine in the presence of a racemic mixture of sec-butylamine.